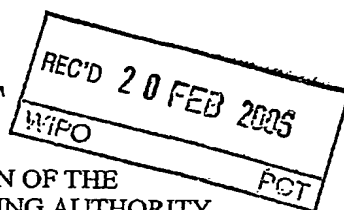


# PATENT COOPERATION TREATY

From the  
INTERNATIONAL SEARCHING AUTHORITY

To:  
G.E.EHRLICH  
G.E. EHRLICH (1995) LTD.  
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**PCT**



WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

Applicant's or agent's file reference <b>27558</b>		Date of mailing (day/month/year) <b>17 FEB 2006</b> <b>FOR FURTHER ACTION</b> See paragraph 2 below
International application No. <b>PCT/IL04/00181</b>	International filing date (day/month/year) <b>24 February 2004 (24.02.2004)</b>	Priority date (day/month/year) <b>27 April 2003 (27.04.2003)</b>
International Patent Classification (IPC) or both national classification and IPC <b>IPC(8): C12P 21/06; C12N 9/00, 9/14, 1/12, 1/20, 5/00, 15/00; C07H 21/04; A01H 11/00 and US Cl.: 435/4, 6, 41, 69.1, 183, 195, 252.1, 252.3, 254.1, 320.1, 325, 410.; 536/23.1, 23.4, 23.5; 800/295</b>		
Applicant <b>METABOGAL, LTD</b>		

1. This opinion contains indications relating to the following items:

- ☒ Box No. I      Basis of the opinion
- ☐ Box No. II      Priority
- ☐ Box No. III      Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV      Lack of unity of invention
- ☒ Box No. V      Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI      Certain documents cited
- ☐ Box No. VII      Certain defects in the international application
- ☐ Box No. VIII      Certain observations on the international application

## 2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/ US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201	Date of completion of this opinion <b>15 November 2005 (15.11.2005)</b>	Authorized officer  Manjunath N. Rao, Ph.D. Telephone No. 571-272-1600
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WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/IL04/00181

Box No. I Basis of this opinion

1. With regard to the language, this opinion has been established on the basis of:

- ☒ the international application in the language in which it was filed  
☐ a translation of the international application into \_\_\_\_\_, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

a. type of material

- ☒ a sequence listing  
☐ table(s) related to the sequence listing

b. format of material

- ☒ on paper  
☒ in electronic form

c. time of filing/furnishing

- ☒ contained in the international application as filed.  
☒ filed together with the international application in electronic form.  
☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

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Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit:
- ☒ paid additional fees
  - ☐ paid additional fees under protest and, where applicable, the protest fee
  - ☐ paid additional fees under protest but the applicable protest fee was not paid
  - ☐ not paid additional fees
2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is
- ☐ complied with
  - ☒ not complied with for the following reasons:  
See the lack of unity section of the International Search Report (Form PCT/ISA/210)

4. Consequently, this opinion has been established in respect of the following parts of the international application:

- ☐ all parts.
- ☒ the parts relating to claims Nos. 1-24, 28-31, 33-37 and 42

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**Box No. V Reasoned statement under Rule 43 bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims <u>4, 12-24, 42</u>	YES
	Claims <u>1-3, 5-11, 28-31, 33-37</u>	NO
Inventive step (IS)	Claims <u>NONE</u>	YES
	Claims <u>1-24, 28-31, 33-37, 42</u>	NO
Industrial applicability (IA)	Claims <u>1-24, 28-31, 33-37, 42</u>	YES
	Claims <u>NONE</u>	NO

2. Citations and explanations:

Please See Continuation Sheet

WRITTEN OPINION OF THE  
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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

V. 2. Citations and Explanations:

Claims 1-3, 5-11, 28-31, 33-37 lack novelty under PCT Article 33(2) as being anticipated by Martin et al. (DNA, 1988, Vol. 7, No.2, pages 99-106). Claims 1-3, 5-11, 28-31, 33-37 are drawn to a host cell producing a high mannose recombinant protein comprising a polynucleotide encoding the recombinant protein and a signal for causing the recombinant protein to be produced as a high mannose protein, wherein the polynucleotide comprises a first nucleic acid sequence encoding said protein of interest operably linked to a second nucleic acid sequence encoding a signal peptide wherein said signal peptide comprises a ER targeting peptide and wherein said host cell is a prokaryotic or a eukaryotic host cell and wherein said polypeptide is one of the lysosomal proteins such as glucocerebrosidase. Claim 28-31, 33-37 are also drawn to a recombinant biologically active high mannose lysosomal enzyme having at least one oligosaccharide chain comprising an exposed mannose residue. Martin et al. disclose one such host cell comprising a polynucleotide encoding said enzyme wherein said polypeptide is produced as a high-mannose protein in high levels. Martin et al. also disclose a recombinant glucocerebrosidase wherein said enzyme is inherently a biologically active high mannose lysosomal enzyme having at least one oligosaccharide chain comprising an exposed mannose residue. Thus, Martin et al. anticipate claims 1-3, 5-11, 28-31, 33-37 as written.

Claims 4, 12-24 and 42 lack an inventive step under PCT Article 33(3) as being obvious over the prior art as applied in the immediately preceding paragraph and further in view of Boller et al. and Zhu et al. Claims 4, 12-24 and 42 are drawn to a host cell producing a high mannose recombinant protein comprising a polynucleotide encoding the recombinant protein and a signal for causing the recombinant protein to be produced as a high mannose protein, wherein the polynucleotide comprises a first nucleic acid sequence encoding said protein of interest operably linked to a second nucleic acid sequence with SEQ ID NO:1 encoding a signal peptide wherein said signal peptide comprises a ER targeting peptide and wherein said polynucleotide is operably linked to a third polynucleotide sequence with SEQ ID NO:2 encoding a plant vacuolar targeting sequence, and wherein said host cell is a plant cell and wherein said polypeptide is one of the lysosomal proteins such as glucocerebrosidase. Claim 42 is drawn to a recombinant protein produced from a plant host cell. The reference of Martin et al. has already been discussed above. Martin et al. teach the production of glucocerebrosidase, a lysosomal protein recombinantly using a host cell comprising a polynucleotide with a signal sequence. The reference of Zhu et al. teach the polynucleotide encoding the signal peptide SEQ ID NO:1 and its use in producing novel recombinant proteins. On similar lines Boller et al. teach the vacuolar targeting sequence SEQ ID NO:2 and its use in targeting polypeptides into the vacuolar space. The invention as a whole is directed to production of glucocerebrosidase as a transgenic protein in plant host cells. The art and the above references teach and provide all sequences required for expressing the glucocerebrosidase as a transgenic protein. The production of mammalian proteins in plant products such as fruits and seed is well known since it eliminates the steps of purification and makes the recombinant protein ready for administration as a plant product. Therefore, with the above references in hand, it would have been obvious to one of ordinary skill in the art to produce human glucocerebrosidase, which is used in enzyme replacement therapy for lysosomal enzyme disorders, as a plant protein by expressing as a polynucleotide linked to the above signal sequence and vacuolar targeting sequences. One of ordinary skill in the art would have been motivated to do so since the lysosomal protein is extensively used in enzyme replacement therapy and production of the protein as a plant product would avoid the extensive purification steps and can be easily administered as a plant

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**Supplemental Box**

In case the space in any of the preceding boxes is not sufficient.

product. One of ordinary skill in the art would have had a reasonable expectation of success since Martin et al. already provide a host cell producing the high-mannose protein, Zhu et al. and Boller et al. provide the sequences to make a DNA construct to be expressed in a plant cell. Therefore the above invention would have been *prima facie* obvious to one of ordinary skill in the art.

Claims 1-24, 28-31, 33-37, 42 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.